Article

Expression of IL-1α, IL-6, TGF-β, FasL and ZNF265 During Sertoli Cell Infection by Ureaplasma Urealyticum

Rongping Li[1], Yebin Xi[1], Xiuzhi Liu[1], Guangjie Chen[1], Baoguo Wang[1], Lihua Jiang[1] and Weiyi Li[1, 2]

To investigate immunoregulatory mechanisms of Sertoli cells in the testis *in vitro* and *in vivo,* we utilized our well-characterized Ureaplasma Urealyticum (UU)-induced model. We investigated the expressions of IL-1α, IL-6, TGF-β, FasL and ZNF265 at the first, second and third weeks post-infection. During recovery from inflammation and with the help of negative regulators TGF-β and FasL, the high levels of IL-1α and IL-6 expressions were observed in the early stages of the infection, and decreased gradually in the later weeks both *in vitro* and *in vivo*. The trend of varied expression of ZNF265 was similar to those of TGF-β and FasL *in vitro* and *in vivo* for Sertoli cells infected with UU. *Cellular & Molecular Immunology*. 2009;6(3):215-221.

Key Words: ZNF265, TGF-β, FasL, Sertoli cell, immunoregulation

Introduction

The mammalian testis is composed of the seminiferous tubules and the interstitium. Sertoli cells within the seminiferous tubules play a crucial role in immunoregulatory function during infection of the testis by invading pathogenic microorganisms, such as Ureaplasma Urealyticum (UU), Uropathogenic *Escherichia coli.* Anatomically, the seminiferous epithelium is a continuation of the urethra. The excurrent ducts provide a gateway for ascending microbial infections that can manifest themselves as urethritis, prostatitis, epididymitis, or more often as combined epididymo-orchitis. It is therefore not surprising that in 13-15% of all cases of male infertility, infection and inflammation of the genital tract are causes or cofactors. Responding to the inflammation of the testis, Sertoli cells play their regulatory role *via* secretion of a series of cytokines (1).

Studies have shown that the constitutive expression of Fas ligand (FasL) on Sertoli cells can induce the apoptosis of migrating immune and inflammatory cells (2). However, more recent studies by others have generated contradictory data and indicated a paradoxical role of FasL in immune privilege. The explanation for immune privilege has later been expanded to include immune deviation and cytokines (3, 4). It has been shown that co-expression of transforming growth factor-β (TGF-β) and FasL promote immune tolerance and protection, preventing inflammation of allografts (5). Additionally, TGF-β appears to play a critical regulatory role by inhibiting specific immune responses, limiting the aggravation of testis inflammation after infection.

It has been demonstrated that Sertoli cells constitute an important source of interleukin-1α (IL-1α) and IL-6 within the testis. Both of these are generally known to be key pro-inflammatory cytokines in inflammation (6, 7). In addition to their involvement in inflammatory events, IL-1α and IL-6 are also most probably involved in local control of spermatogenesis. In fact, IL-1α was found to stimulate DNA replication in mitotic spermatogonia and in meiotic spermatocytes *in vitro* (8). Other *in vitro* experiments have shown that IL-6 has the opposite effect (9). Expression of immunosuppressive cytokine TGF-β in the Sertoli cells can inhibit a number of pro-inflammatory activities of macrophages and lymphocytes, including the production and/or actions of IL-1α, TNF-α and IL-6 (10).

ZNF265 (formally termed "Zis") is an arginine/serine-rich (RS) domain containing zinc finger protein. Studies show that ZNF265 proteins are co-immunoprecipitated with mRNAs and colocalize with splicing factors SMN, U1-70K, U2AF35 and SC35, indicating that ZNF265 is a novel component of spliceosomes (11, 12). It has been known that many zinc finger proteins such as Gfi, GATA3, Egr-1, Aiolos, ZNF569 (13, 14) play a crucial role in the immunoregulatory function of animals.

In our recent research, ZNF265 proteins were shown to be strongly expressed in the Sertoli cells. This work and findings of the Sertoli cells's notable immunoregulatory

[1]Shanghai Jiao Tong University School of Medicine, Department of Immunology, Shanghai Institute of Immunology, Shanghai 200025, China;

[2]Correspondence to: Dr. Weiyi Li, Shanghai Jiao Tong University School of Medicine, Department of Immunology, Shanghai Institute of Immunology, Shanghai 200025, China. Tel: +86-21-6384-6590-776-793, E-mail: liweiyi@sjtu.edu.cn

Received Feb 21, 2009. Accepted Apr 27, 2009.

function suggest continued study examining the immuno-regulatory mechanisms of Sertoli cells in the testis and the change in expression of ZNF265 during infection of Sertoli cells with UU. We instituted these studies using our well-characterized UU induced model (15).

Material and Methods

Animals

The animals used were 2-week-old and 5 to 7-week-old male Sprague-Dawley (SD) rats. Animals were purchased from Shanghai Laboratory Animal Center of the Chinese Academy of Sciences (China) and kept at the Animal Center of Shanghai Jiao Tong University. All rats were bred and maintained in specific pathogen-free conditions. Animal experimental procedures were in compliance with institutional guidelines.

Reagents

The primary antibody, rabbit anti-rat ZNF265, was donated by professor Ping Xu of the Department of Science at Shanghai Fu Dan University. Fluorescein labeled goat anti-rabbit IgG (KPL, Maryland USA, 02-15-06), biotin anti-rat FasL (106703) and streptavidin-FITC (405202) were purchased from Biolegend (USA). Rat TGF-β (BMS623), IL-1α (BMS627), an ELISA kit and rat IL-6 module set (BMS213) were purchased from Bender MedSystems (Vienna, Austia, Europe). Collagenase type II (47D9570) and hyaluronidase (P5C7983) were purchased from Worthington Biochemical Corp (USA).

UU culture and titer confirmation

Serum type 8 standard lines (T960) were donated by Dr. Robeston of the Institute of Microbiology and Infection at Canada Alberta University. Standard UU bacteria T960 (freeze-dried product) were reconstituted, then inoculated into UU culture medium (Shanghai Enkang, 0805158) under germfree conditions and incubated for 18-24 h at 37°C.

The cultured UU (as described above) was double diluted in the period of logarithmic growth, inoculated into a 40-well plate for 48 h at 37°C and the result was observed. The titer of UU was expressed as color-changing units (CCU/ml). The maximal dilution of red color, representing the titer of UU, was 1×10^5 CCU/ml.

Sertoli cell culture and infection with UU

Sertoli cells were removed from male SD rats at 2 weeks of age and cultured as previously described (16). Briefly, Sertoli cells were isolated using repeated enzymatic digestions with collagenase type II and hyaluronidase and then cultured in 24-well plates at 5×10^5 cells per well in a volume of 1 ml DMEM/F-12 medium (Sigma, SH30004.01). To remove residual germ cells in the culture and obtain pure Sertoli cells, after 2 days the cultures were subjected to hypotonic shock by incubation at room temperature with 20 mM Tris-HCl buffer (pH 7.4) for 2 min. In all experiments, the monolayers used were composed of at least 92% pure Sertoli cells.

On the second day of culture, the Sertoli cells were infected with UU at a dosage of 200 μl/well, while the control group remained untreated. All samples were incubated at 37°C, 5% CO_2. The supernatants were collected and analyzed for secretory cytokines, while the cells were tested for surface-marker expression by flow cytometric analysis or were lysed for RT-PCR.

Induction of testis partial infection in vivo

The rat testis partial infection operation was proceeded as previously described (15): the urinary bladder of each rat was injected with 0.3 ml UU suspended in culture medium liquid. *In vivo*, male rats (5 to 7-weeks old) were infected while a control group was treated with 0.9% physiological saline. Weekly after the treatment, the rats were sacrificed, and Sertoli cells were isolated and prepared for immunohisto-chemical, flow cytometric and RT-PCR analyses.

ELISA

Culture supernatants isolated from Sertoli cells cultured in treatment groups were collected to measure the secretion of IL-1α, IL-6 and TGF-β using the specific ELISA assay according to manufacturer's instruction. The results were analyzed using the SOFTmaxPRO analytical system.

Immunohistochemistry

Rat testis samples were subjected to routine deparaffinization and rehydration. Antigen retrieval was achieved by microwaving in 0.01 mol/L citrate buffer for 10 min and then cooling for 30 min. The endogenous peroxidase activity was inhibited by incubation with 3% hydrogen peroxide in methanol for 20 min and nonspecific binding was blocked by incubation with 5% goat serum in phosphate-buffered saline

Table 1. Primer sequences and PCR conditions for cDNAs

cDNA	5' primer	3' primer	Product size	Cycles
IL-1α	GACCATCTGTCTCTGAATCAG	CGATGAGTAGGCATACATGTC	453 bp	34
IL-6	CTAGGAAGAACTGGCAATATG	AAACCATCTGGCTAGGTAAGA	207 bp	32
TGF-β	CCGCAACAACGCAATCTAT	GAAAGCCCTGTATTCCGTCTC	308 bp	30
FasL	GGAATGGGAAGACACATATGGAACTGC	CATATCTGGCCAGTAGTGCAGTAATTC	238 bp	28
ZNF265	GGGGAACAGAAATAGGAAA	CCCCAAGAAAAAGATCTTA	304 bp	25
β-actin	GACCTTCAACACCCCAGC	ACGCACGATTTCCCTCTC	389 bp	25

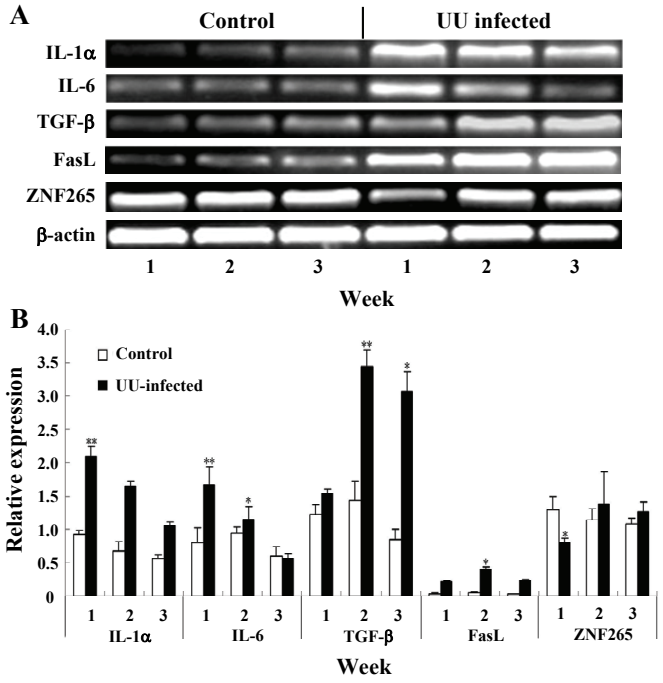

Figure 1. Expressions of IL-1α, IL-6, TGF-β, FasL and ZNF265 mRNA in UU-infected Sertoli cells *in vitro*. (A) Sertoli cells were isolated from SD rats and infected with UU or left untreated as control *in vitro*. Weekly after the treatment, Sertoli cells were collected for RT-PCR analysis. (B) Relative expressions of IL-1α, IL-6, TGF-β, FasL and ZNF265 normalized to β-actin. Data were represented as mean ± SD from four independent experiments, $*p < 0.05$, $**p < 0.01$, *vs* control groups.

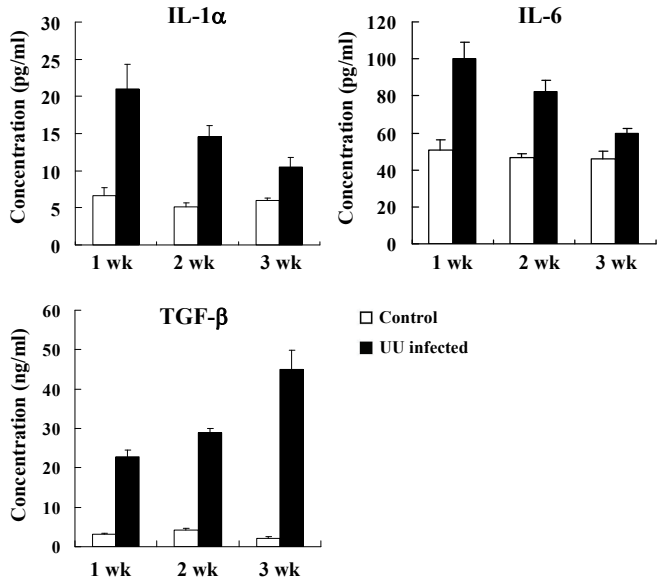

Figure 2. Secretions of IL-1α, IL-6 and TGF-β by Sertoli cells *in vitro*. Sertoli cells were isolated from SD rats and infected with UU or left untreated as control. Weekly after the treatment, the supernatants were collected to measure the expressions of IL-1α, IL-6 and TGF-β using a specific ELISA. Data were represented as mean ± SD from four independent experiments $*p < 0.05$, $**p < 0.01$, *vs* control.

(PBS) at room temperature. After three PBS washes, the specimens were reacted overnight at 4°C with rabbit anti-rat monoclonal antibodies: anti-IL-1α (Santa Cruz, H-159), anti-IL-6 (Santa Cruz, R-19), anti-TGF-β (Santa Cruz, H-112). After incubation with goat anti-rabbit-IgG2b-horseradish peroxidase, signal was developed with 3,3'-diamino-benzidine tetrahydrochloride in Tris-HCl buffer (pH 7.6) containing 0.02% hydrogen peroxide. The sections were then counterstained with hematoxylin and mounted. Negative controls were performed by replacing the specific primary antibody with PBS. The immunolocalization of each antigen was achieved using the immunoperoxidase system. Sertoli cells with membrane or cytoplasma buffy stain were considered to be positive. Positive cells were scored according to the stain intensity: (1) light buffy; (2) heavy buffy; (3) brown. Scores by the pencent of positive Sertoli cells were: 0, 1-25%; 1, 26-50%; 2, 51-75%; 3, 76-100%. Index of labeled Sertoli cells (labeling index, LI) was calculated by the formula: LI = score according to the stain intensity/score by the percent of positive Sertoli cells.

Flow cytometry

For analysis of surface FasL and intracellular ZNF265 expressions, Sertoli cells obtained from *in vivo* or *in vitro* preparations were detached with trypsin and washed with cold PBS containing 1% BSA. For detection of FasL, Sertoli cells were incubated with biotin anti-rat FasL for 40 min at 4°C then incubated with streptavidin-FITC for 25 min at 4°C after washing twice. For detection of ZNF265, a Fixation/Permeabilization kit (eBioscience) was used to fix and permeabilize the Sertoli cells (4°C, 60 min). Cells were then incubated with rabbit anti-rat ZNF265 (4°C, 40 min) and stained with fluorescein labeled goat anti-rabbit IgG (4°C, 25 min). The results were analyzed using FACSCalibur (BD).

RT-PCR

Total RNA was extracted from Sertoli cells using TRIzol reagent (Invitrogen). The expressions of IL-1α, IL-6, TGF-β, FasL and ZNF265 in isolated Sertoli cells from each group were analyzed. Primer pairs specific for rat IL-1α, IL-6, TGF-β, FasL and ZNF265 cDNAs were designed using published rat cDNA sequences (Table 1).

PCR-expanded products of β-actin, IL-1α, IL-6, TGF-β, FasL and ZNF265 from each group were resolved on 1.5% polyacrylamide gel electrophoresis using 1.5 V/cm, 0.5× TBE buffer. The PCR products were analyzed using BandScan 4.3 software (Glyko, Novato, CA, USA) to achieve intermediate values and the ratios of IL-1α/β-actin, IL-6/β-actin, TGF-β/β-actin, FasL/β-actin and ZNF265/β-actin for each group were calculated to represent the expressions of IL-1α, IL-6, TGF-β, FasL and ZNF265.

Statistical analysis

The *t*-test using SPSS 11.0.1 software was applied to

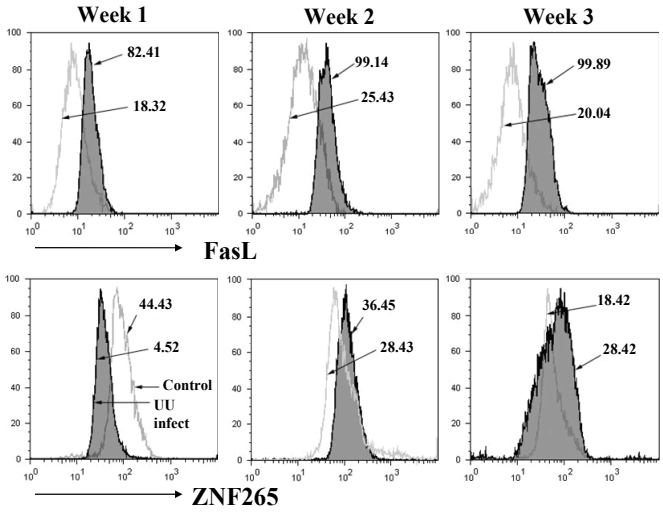

Figure 3. Expressions of surface FasL and intracellular ZNF265 in UU-infected Sertoli cells *in vitro*. Sertoli cells were isolated from SD rats and infected with UU or left untreated as control. Weekly after the treatment, Sertoli cells were collected to analyze the expressions of FasL and ZNF265 by flow cytometry. The number indicated the value of mean fluorescence intensity (MFI). Data were shown as representation of four independent experiments.

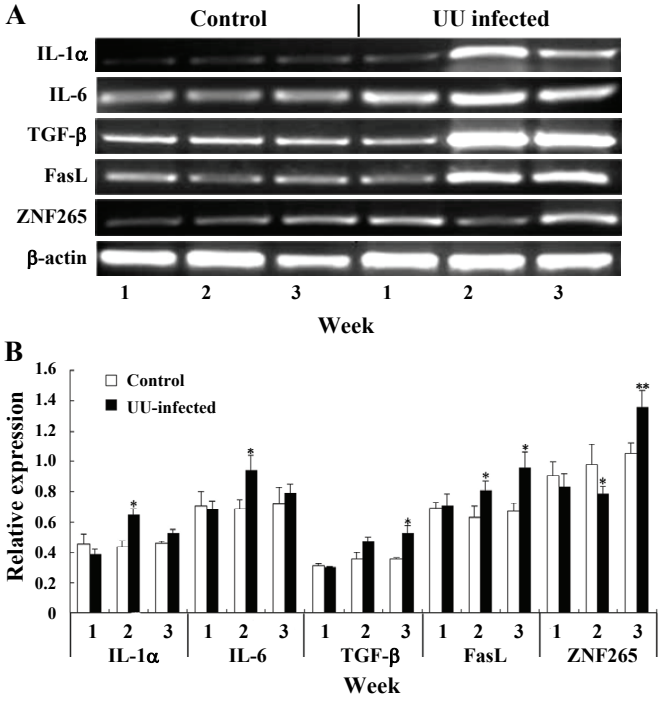

Figure 4. Expressions of IL-1α, IL-6, TGF-β, FasL and ZNF265 mRNA in testis Sertoli cells *in vivo*. (A) The rats were infected with UU (infected group) or treated with 0.9% physiological saline (control group). Weekly after the treatment, Sertoli cells in testis were isolated for RT-PCR. (B) Relative expressions of IL-1α, IL-6, TGF-β, FasL and ZNF265 normalized to β-actin. Data were represented as mean ± SD from four independent experiments, *$p < 0.05$, **$p < 0.01$, *vs* control groups.

compare two samples' averages for statistical significance in all experiments. Values of $p < 0.05$ were considered statistically significant.

Results

IL-1α, IL-6, TGF-β, FasL and ZNF265 mRNA expressions in UU-infected Sertoli cells in vitro

Sertoli cells were infected with UU or not and the mRNA expressions of IL-1α, IL-6, TGF-β, FasL and ZNF265 were assayed by semi-quantitive PCR. The levels of IL-1α and IL-6 in UU-infected Sertoli cells showed a significant increase compared with those of the control after 1 week post-infection, and decreased gradually after 2 weeks. The expressions of FasL and TGF-β mRNAs showed no significant difference after 1 week, but increased gradually after 2 weeks compared with control. While ZNF265 in UU-infected Sertoli cells decreased after the 1st week, and increased after 2 weeks and maintained a parallel level with control (Figure 1).

IL-1α, IL-6, TGF-β, FasL and ZNF265 expressions at protein levels in UU-infected Sertoli cells in vitro

As shown in Figures 2 and 3, the trends of IL-1α, IL-6, and ZNF265 expressions were similar to those of their mRNA expressions as shown above. The expressions of FasL and TGF-β increased after 1 week. In the following 2 weeks, FasL expression remained at the same levels, while TGF-β increased gradually compared with controls.

IL-1α, IL-6, TGF-β, FasL and ZNF265 mRNA expressions in UU-infected Sertoli cells in vivo

The rats were infected with UU (infected group) or treated with 0.9% physiological saline as control group. Weekly after the treatment, Sertoli cells in testis were isolated for RT-PCR analysis. As shown in Figure 4, the expressions of IL-1α and IL-6 did not increase until the 2nd week, and decreased after 3 weeks in Sertoli cells of the UU-infected group compared with control group. TGF-β increased significantly after 3 weeks and the expression of FasL increased gradually over the 2nd and 3rd weeks.

ZNF265 in UU-infected rat Sertoli cells, compared with control, showed no significant difference after 1 week, but decreased at the end of the 2nd week and increased again after 3 weeks (Figure 4). That the expressions of IL-1α, IL-6, TGF-β, FasL and ZNF265 mRNAs did not show significant difference after 1 week possibly because of the distance from the urinary bladder to the testis preventing UU infection during the 1st week.

The expressions of IL-1α, IL-6, TGF-β, FasL and ZNF265 at protein levels in UU-infected Sertoli cells in vivo

Sertoli cells in testis were isolated from rats infected with UU or treated with 0.9% physiological saline and analyzed the

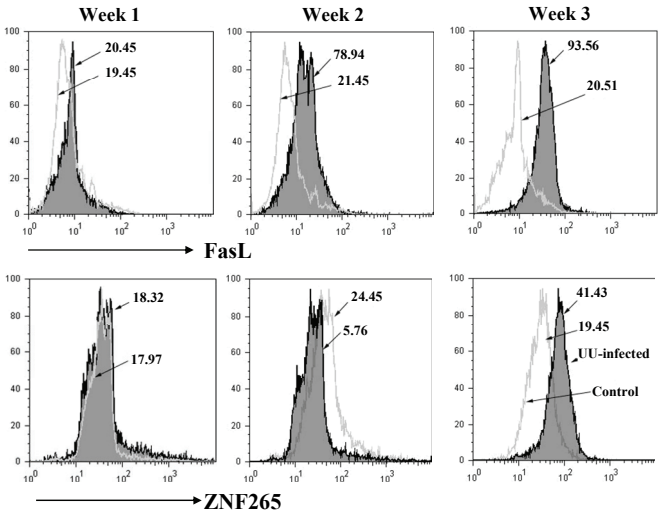

Figure 5. Expressions of surface FasL and intracellular ZNF265 in Sertoli cells of rat testis after 3 weeks post UU-infection *in vivo*. Sertoli cells in testis were isolated from rats infected with UU (infected group) or treated with 0.9% physiological saline (control group) and analyzed the expressions of FasL and ZNF265 by flow cytometry. The number indicated the value of mean fluorescence intensity (MFI). Data were shown as representation of four independent experiments.

expressions of FasL and ZNF265 by flow cytometry. The results showed that the trends of FasL and ZNF265 expressions were similar to those of their corresponding mRNA *in vivo* (Figure 5). The protein expressions of IL-1α, IL-6 and TGF-β in testis tissues were examined by immunohistochemistry. As shown in Figure 6 and Table 2, the expressions of IL-1α and IL-6 were upregulated after 2 weeks post-infection with a peak value after 2 weeks. And TGF-β showed a significant increase after 2 weeks compared with control.

Discussion

It is well established that bacterial and viral infections of the male reproductive tract contribute significantly to impaired fertility. The direct pathologic effects resulting from the bacterial invasion are often aggravated by the inflammatory process. Sertoli cells play a crucially regulatory role in controlling the transformation of inflammation. ZNF265, a novel spliceosomal protein able to induce alternative splicing, is an arginine/serine-rich (RS) domain containing zinc finger protein (11) and shows notable expression in Sertoli cells. As Sertoli cells have a special immunoregulatory function, it is important to understand the mechanisms involved in regulating inflammation initiated against bacteria. We used the UU-induced model (15) to reveal the immunoregulatory function mechanism of Sertoli cells and to explore the changes in ZNF265 expression when Sertoli cells become infected. The inflammation of the testis was provoked at the second week after the urinary bladder was injected with UU,

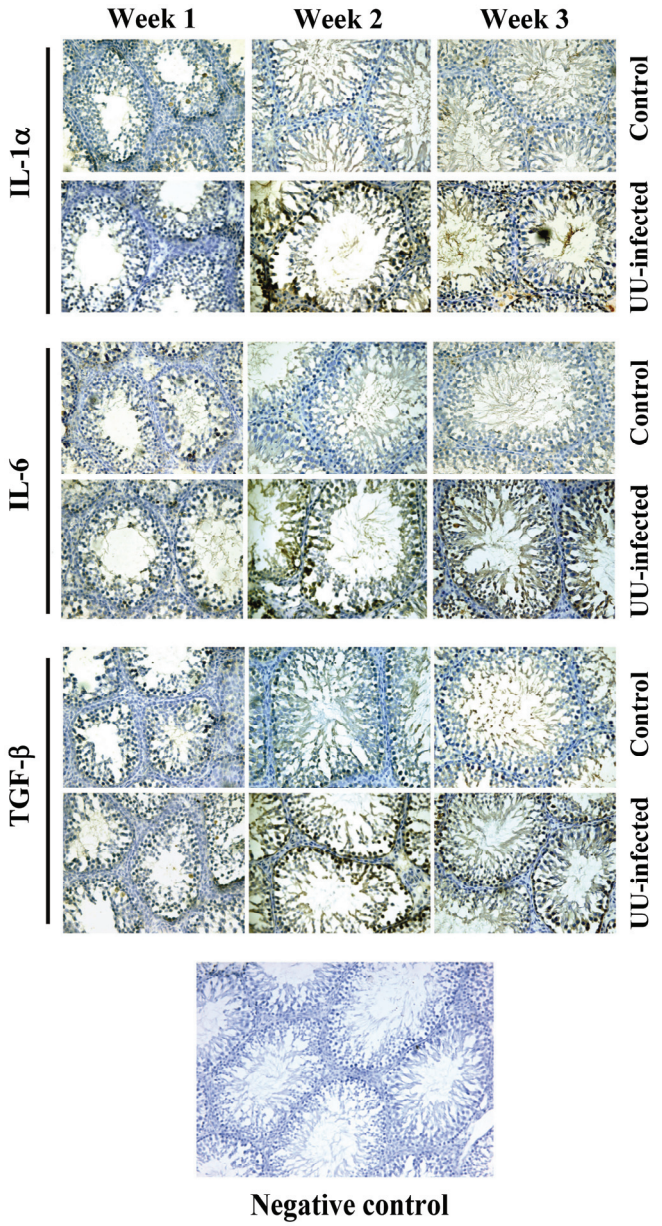

Figure 6. The expressions of IL-1α, IL-6 and TGF-β in testis by immunohistochemistry performed on seminiferous tubules. The rats were infected with UU (infected group) or treated with 0.9% physiological saline (control group). Weekly after the treatment, testis samples were collected from each rat and the expressions of L-1α, IL-6 and TGF-β were analyzed. (Magnification: ×400).

because of the distance from the urinary bladder to the testis *in vivo*. However, inflammation was noted at the first week *in vitro*.

In this study we found that the tendency of ZNF265 expression in the UU-infected group as compared to the control group was similar to that of TGF-β, both *in vitro* and *vivo,* showing a slight decrease in the early stage of inflammation and upregulated expression at later weeks. However, the range of ZNF265 expression was lower than

Table 2. The comparison of IL-1α, IL-6, TGF-β in rat testis after partial infected by UU

Group	Protein	Time		
		Week 1	Week 2	Week 3
Control	IL-1α	2.58 ± 0.44	1.94 ± 0.68	2.26 ± 0.26
	IL-6	2.47 ± 0.39	3.03 ± 0.36	2.85 ± 0.68
	TGF-β	1.62 ± 0.24	1.58 ± 0.30	1.50 ± 0.12
UU infected	IL-1α	1.59 ± 0.39	7.46 ± 0.86**	4.87 ± 0.67*
	IL-6	3.46 ± 0.26	8.74 ± 0.86**	6.38 ± 0.83*
	TGF-β	1.28 ± 0.14	5.68 ± 0.63*	8.46 ± 0.95**

Testis samples from each animal *in vivo* were examined by immunohistochemistry. The number of positive cells in each section was calculated by counting the number of TUNEL-positive cells in 10 fields per slide at ×400 magnification. Data were given as mean ± SD from four independent experiments, *$p < 0.05$, **$p < 0.01$, *vs* control.

that of TGF-β. The expression of immunosuppressive cytokine TGF-β has been observed in isolated Sertoli cells (17). TGF-β, a secreted and cell-associated polypeptide with a immunosuppressive function in innate and adaptive immunity results in failure to recognize or respond adequately to self, foreign, or tumor-associated antigens and inhibits the production and/or actions of pro-inflammatory cytokines, including IL-1α, TNF-α, and IL-6 (10, 18). Our results have shown that either mRNA levels or protein levels of TGF-β, both *in vitro* and *in vivo*, accompany the rise of TGF-β at the second and third weeks. Further, high levels of IL-1α and IL-6 in the UU-infected group, as compared to the control group at the first week, showed a significant down-regulation at the second and third weeks. It has been demonstrated that the high level of TGF-β at the later second and third weeks could have educed the immunosuppressive function *via* inhibition of the secretion of pro-inflammatory cytokines IL-1α and IL-6. The expressions of ZNF265 during the second and third weeks were increased, perhaps in an effort to control the inflammation or maintain the normal immunoregulatory function of the Sertoli cells. However, further experiments are needed to confirm this.

IL-1α and IL-6 are pro-inflammatory cytokines. IL-1α is present in the testis under normal homeostatic circumstances and increase upon infection (19). It has recently been demonstrated that IL-1α expression and production by Sertoli cells are triggered by phagocytosis of residual bodies originating from late spermatids (7, 20) at the time of spermiation and that this cytokine is able to stimulate Sertoli cells secreting IL-6 by an autocrine mechanism (21). IL-1α enhances inflammation by promoting NK, T cell and macrophage proliferation and differentiation, recruiting macrophages/monocytes for its chemotaxis. When FasL induces apoptosis of Fas positive cells, it can active caspase-mediated pathways which can secrete IL-1α. IL-6 stimulates lymphocyte activation and proliferation. However, it also upregulates the acute phase response and stimulates production of anti-inflammatory cytokines by T cells (22). In order to resist pathogenic microorganism UU, IL-6 appears to cooperate with IL-1α to strengthen the local inflammatory reaction directly or through affecting some immune cells' response in testis during the early infection. Thus, we could see that IL-1α and IL-6 were upregulated notably at the first week *in vitro* and at the second week *in vivo* (the early stage of the inflammation). As the inflammation took a favorable turn and with the help of negative regulation by cytokine TGF-β, the levels of IL-1α and IL-6 expressions decreased gradually in the later weeks both *in vitro* and *in vivo*. In the initial stage of the inflammation of Sertoli cells, the incremental changes for IL-1α and IL-6 *in vivo* were lower than those *in vitro*, because the testis *in vivo* has a complex internal environment. Sertoli cells, together with other cells and tissue structures, strengthen the immunoloregulation of inflammation against UU.

Fas/FasL (APO-1/APO-1L; CD95/CD95L) system is an evolutionarily highly conserved biological mechanism involving the deletion and renewal of cells important for the maintenance of immune privilege. Studies have clearly demonstrated the ability of FasL to protect allogeneic grafts from immune rejection and to sustain immune tolerance in an immune-privileged site such as the testis (23, 24). Our results showed that the level of FasL was up-regulated from the first to the third weeks *in vitro* and at the second and third weeks *in vivo* during Sertoli cell infection with UU, while the control group values did not change. The Fas system is a mechanism through which cells expressing FasL induce apoptosis of Fas expressing cells. Fas is abundantly expressed in various tissues, particularly in activated T and B cells. FasL expressed on the cell surface induced by UU infection plays a key role in eliminating T-cell populations following antigenic (UU) stimulation and clonal proliferation *via* apoptosis as mediated by Fas, thereby down-regulating the immune response in testis (25, 26). We propose that FasL, together with TGF-β, promotes immunoregulation in preventing inflammation at the later second and third weeks; IL-1α and IL-6 increase in the early stages of the infection in an attempt to eradicate the pathogen; ZNF265 increases during the second and third weeks, perhaps in an effort to control the inflammation or maintain the normal immuno-regulatory function of Sertoli cells in the testis.

In order to confirm the immunoregulatory function of ZNF265 in Sertoli cells, RNAi or gene knockout technology

will be used to suppress the expression of ZNF265 in upcoming studies in our laboratory.

Acknowledgements

This study was financially supported by the key innovative project of the ShangHai educational committee (No. 09ZZ117) and Shanghai institute of Immunology Science Foundation (No. 08A05) We thank Professor Ping Xu (Institute of Life Science, Fu Dan University) for the gift of primary antibody, rabbit anti-rat ZNF265.

References

1. Fijak M, Menhardt A. The testis in immune privilege. Immunol Rev. 2006;213:66-81.
2. Bellgrau D, Gold D, Selawry H, et al. A role for CD95 ligand in preventing graft rejection. Nature. 1995;377:630-632.
3. Restifo NP. Not so Fas: re-evaluating the mechanisms of immune privilege and tumor escape. Nat Med. 2000;6:493-495.
4. Green DR, Ferguson TA. The role of Fas ligand in immune privilege. Nat Rev Mol Cell Biol. 2001;2:917-924.
5. Chen JJ, Sun Y, Nabel GJ. Regulation of the proinflammatory effects of Fas ligand (CD95L). Science. 1998;27:282:1714-1717.
6. Khan SA, Söder O, Syed V, et al. The rat testis produces large amounts of an interleukin-1-like factor. Int J Androl. 1987;10: 495-503.
7. Syed V, Ge´rard N, Kaipia A, et al. Identification, ontogeny and regulation of an interleukin-6-like factor in the rat seminiferous tubule. Endocrinology. 1993;132:293-299.
8. Rozwadowska N, Fiszer D, Jedrzejczak P, et al. Interleukin-1 superfamily genes expression in normal or impaired human spermatogenesis. Genes Immun. 2007;8:100-107.
9. Hakovirta H, Syed V, Je´gou B, et al. Function of interleukin-6 as an inhibitor of meiotic DNA synthesis in the rat seminiferous epithelium. Mol Cell Endocrinol. 1995;108:193-198.
10. Li MO, Wan YY, Sanjabi S, et al. Transforming growth factor-β regulation of immune responses. Ann Rev Immunol. 2006;24: 99-146.
11. Adams DJ, Van der Weyden L, Mayeda A, et al. ZNF265-α novel spliceosomal protein able to induce alternative splicing. J Cell Biol. 2001;154:25-32.
12. Li J, Chen XH, Xiao PJ, et al. Expression pattern and splicing function of mouse ZNF265. Neurochem Res. 2008;33:483-489.
13. Karsunky H, Mende I, Schmidt T, et al. High levels of the onco-protein Gfi-1 accelerate T-cell proliferation andinhibit activation induced T-cell death in Jurkat T-cells. Oncogene. 2002;21:1571-1579.
14. Ho IC, Pai SY. GATA-3 not just for Th2 cells anymore. Cell Mol Immunol. 2007;4:15-29.
15. Xu C, Wang YF, Sun GF. The creation of UU infected animal model. Sheng Zhi Yu Bi Yun. 1995;15:205-209.
16. Galdieri M, Ziparo E, Palombi F, et al. Pure Sertoli cell cultures: new model for the study of somatic-germ cell interaction. J Androl. 1981;5:249-254.
17. Mullaney BP, Skinner MK. Transforming growth factor-β (β1, β2, and β3) gene expression and action during pubertal development of the seminiferous tubule: potential role at the onset of spermatogenesis. Mol Endocrinol. 1993;7:67-76.
18. Wahl SM, Wen J, Moutsopoulos N. TGF-β: a mobile purveyor of immune privilege. Immunol Rev. 2006;213:213-227.
19. Ganaiem M, Abuelhija M, Lunenfeld E, et al. Effect of interleukin-1 receptor antagonist gene deletion on male mouse fertility. Endocrinology. 2009;150:295-303.
20. Gérard N, Syed V, Jégou B. Lipopolysaccharide, latex beads and residual bodies are potent activators of Sertoli cell interleukin-1α production. Biochem Biophys Res Commun. 1992;185:154-161.
21. Syed V, Stéphan JP, Gérard N, et al. Residual bodies activate Sertoli cell interleukin-1α (IL-1α) release, which triggers IL-6 production by an autocrine mechanism, through the lipoxygenase pathway. Endocrinology. 1995;136:3070-3078.
22. Tilg H, Dinarello CA, Mier JW. IL-6 and APPs: anti-inflammatory and immunosuppressive mediators. Immunology. 1997;18:428-432.
23. Bohana-Kashtan O, Civin CI. Fas ligand as a tool for immuno-suppression and generation of immune tolerance. Stem Cells. 2004;22:908-924.
24. Chen JJ, Sun Y, Nabel GJ. Regulation of the proinflammatory effects of Fas ligand (CD95L). Science. 1998;27:1714-1717.
25. Riccioli A, Salvati L, D'Alessio A, et al. The Fas system in the seminiferous epithelium and its possible extra-testicular role. Andrologia. 2003;35:64-70.
26. Zhang Y, Xu G, Zhang L, et al. Th17 cells undergo Fas-mediated activation-induced cell death independent of IFN-γ. J Immunol. 2008;181:190-196.